

D0297 NP

Fig. 1A

1	CACTCACACACCTACGGACACACGCTACTCTGGGAGGTGATTGCGACTTAGCCAGGCC	60
61	CCAAAGCTGGGCTCCTGTAGGGAGAAAGTCTGCCAGGTCCACATCCAAGCCTTCATCGT	120
121	TTGTCCTCCGGGTTCTGGGATCCTGCTGGAAGAGGGAGCTTCTGCAATGGGAGTTGCCA	180
1	M G V A T	5
181	CAACCCTGCAGCCCCAACCAACCACTTCCAAAACCTTGCAAGCAGCATCTAGAACAGTGG	240
6	T L Q P P T T S K T L Q K Q H L E A V G	25
241	GCGCCTACCAATATGTGCTCACTTCCTCTTCATGGGCCCTTCTTCTCCCTTCTGTCT	300
26	A Y Q Y V L T F L F M G P F F S L L V F	45
301	TTGTCCTCCTCTCACGTCACTCTGGCCCTCTCTGTTTTACTTGGTGTGGCTATG	360
46	V L L F T S L W P F S V F Y L V W L Y V	65
361	TGGACTGGGACACACCCAACCAAGGTGGAAGGCCTCGGAGTGGATAAGGAACCGGGCAA	420
66	D W D T P N Q G G R R S E W I R N R A I	85
421	TTTGGAGACAACTAAGGGATTATTATCCTGTCAAGCTGGTGAAAACAGCAGAGCTGCC	480
86	W R Q L R D Y Y P V K L V K T A E L P P	105
481	CGGATCGGAACACTACGTGCTGGCGCCACCCCTCATGGATCATGTGTACAGGCTCCT	540
106	D R N Y V L G A H P H G I M C T G F L C	125
541	GTAATTCTCCACCGAGAGCAATGGCTCTCCCAGCTCTCCGGGGCTCCGGCCCTGGT	600
126	N F S T E S N G F S Q L F P G L R P W L	145
601	TAGCCGTGCTGGCTGGCTCTTCTACCTCCGGTCTATCGCACTACATCATGTCTTG	660
146	A V L A G L F Y L P V Y R D Y I M S F G	165
661	GACTCTGTCCGGTGAGCCGCCAGAGCTGGACTTCATCCTGTCCCAGCCCCAGCTCGGGC	720
166	L C P V S R Q S L D F I L S Q P Q L G Q	185
721	AGGCCGTGGTCATCATGGTGGGGGTGCGCACGAGGCCCTGTATTCAAGTCCCCGGGAGC	780
186	A V V I M V G G A H E A L Y S V P G E H	205
781	ACTGCCCTACGCTCCAGAAGCGCAAAGGCTTCGTGCGCTGGCGCTGAGGCACGGGGCGT	840
206	C L T L Q K R K G F V R L A L R H G A S	225
841	CCCTGGTGCCCGTGTACTCCTTGGGAGAATGACATCTTAGACTTAAGGCTTTGCCA	900
226	L V P V Y S F G E N D I F R L K A F A T	245
901	CAGGCTCCTGGCAGCATTGGTGCCAGCTCACCTCAAGAAGCTCATGGCCTCTCCTT	960
246	G S W Q H W C Q L T F K K L M G F S P C	265
961	GCATCTCTGGGGTCGGCTCTCTCAGCCACCTCCTGGGGCCTGCTGCCCTTGCTG	1020
266	I F W G R G L F S A T S W G L L P F A V	285

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Fig. 1B

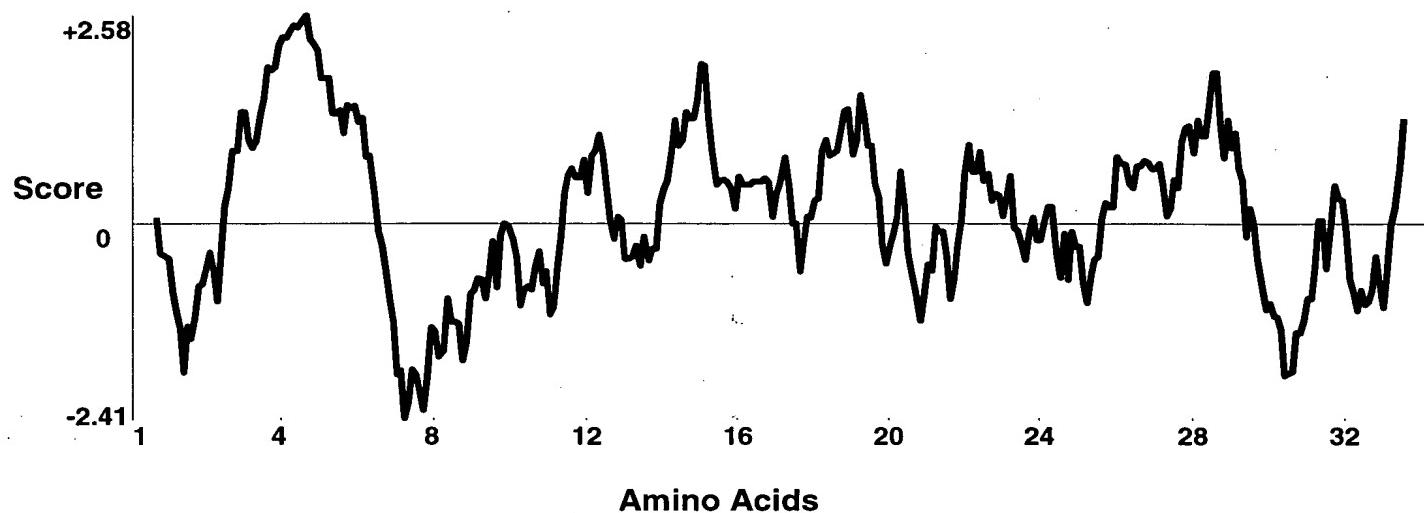
1021	TGCCCATCACCACTGTGGTGGGCCGCCCCATCCCGTCCCCCAGCGCCTCCACCCCACCG	1080
286	P I T T V V G R P I P V P Q R L H P T E	305
1081	AGGAGGAAGTCAATCACTATCACGCCCTCTACATGACGGCCCTGGAGCAGCTTCGAGG	1140
306	E E V N H Y H A L Y M T A L E Q L F E E	325
1141	AGCACAAAGGAAAGCTGTGGGTCCCCGCTTCCACCTGCCTCACCTTCATCTAGGCCTGGC	1200
326	H K E S C G V P A S T C L T F I	341
1201	CGCGGCCTTCGCTGAGCCCTGAGCCAAGGCACTGAGACCTCCACCCACTGTGGACTC	1260
1261	CATGCCTCCAATAAAAGGTAGTTCTGGGCCAGCGCAGTGCCTCGTGCCTGTGATCCCAG	1320
1321	CACTTGGGAGGCCAGGGTGGGAGGATCGTTGAGCCCAGGAGTTGAAGACCAGCCTGGG	1380
1381	CAACACAGTGAGACTTCATTCTACAAAAAAAAAAAAAA	1420

Fig.2: Alignment of Predicted Human MGAT3 with its Homologues

MGAT3 MGAT1 DGAT2	1 (1) ----- (1) ----- (1) MKTLIAAYSGVLRGERQAEADRSQRSHGGPALSREGSGRWGTGSSILSAL	50 -MGV
MGAT3 MGAT1 DGAT2	51 (4) ATTLQPPTTSKTLQKQH LEAVGAYQYVLTFMGPFFSLLVFVLLF T SLW (1) -MKVEFAP L N-IQLARR L QTAVL L OWL S LTGPM SIGIT V MLIHN-YL (51) QDLFSVTWLNRSKVEKQ L QVISV L OWL S FLVLGVAC SAILMYIFCTDCW	100
MGAT3 MGAT1 DGAT2	101 (54) PFSV F YLWLYVWD D TPNQGGRRSEWIRNRAIWRQLRDYYPVKL V KTAEL (48) FLYIP P YLMWL Y FDWHTPERGGRRSSWIKNWTLWKHFKDYFP I HLIK T QDL (101) LIA M LYFTWL V EDWNTPKKGGRRSQWVRNWAVWRYFRDYFP T OLVKTHNL	150
MGAT3 MGAT1 DGAT2	151 (104) P E DRNYVLGAHPHGIMCTGFLCNFSTESNGFSQ I FPGLRPWLA V LAGLFY (98) D P SHNYIEGF H PHGIMAVGAFCNFSVNYSDFKD I FPGFTSYLHVPLWF W (151) LTT R NYIEGY H PHGIMGLGAFCNFSTEATEVSKK F PGIRPYLATLAGNFR	200
MGAT3 MGAT1 DGAT2	201 (154) I P VYRDYIMSFGLCPVSRQSLDFILSQ P QLGQAVVIVMVGGAHEALY S VP G (148) CPVFREYVMSV C EVSVSKKS V SYMVSKEGGGNISV I VLGGA E SLDAH P (201) MPV L REYLMMSGC I CPVSRDTIDYLLSKNGSGNAII I VVGGAAB S LSSMPG	250
MGAT3 MGAT1 DGAT2	251 (204) EHCLT I QKRKG F VR I ALRHGA S LVPVY S FGENDIFRLKA F ATGSWQHWCQ (198) KFTL F IRQRKG F V K IALTHGA S LVPV V SGENELFKOTDNPF G SWIRT V Q (251) KNAVTERNRKG F V K ALRHGA D LVP I Y S FGENEVYKQVIEEG G SWGRWQ	300
MGAT3 MGAT1 DGAT2	301 (254) LTFKKLMGFSPCI F WGRGLFSATSW G LLPFAVE I TTVVGRPI P V P ORLHP (248) NKL O KIMGFALPLFHARGVFOYN-FGLMTYRKA I TTVVGRPI P V R OTLNP (301) KKFOKYIGFAPCI F HGRGLFSSDTWGLVPYS K PTTVVGEPI T IPKLEHP	350
MGAT3 MGAT1 DGAT2	351 (304) TEEEVNHYHALYM T ALEQLFEEHKESC G V P A S T C LT F I (297) T O EOT E ELHQTYMEELRKLFEEHK G KYGIPEHETLV K (351) T Q QD I DLYHTM Y MEALVKLF D KHKT K F G L P ETEV L EVN	388

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Fig.3 Hydrophobicity Analysis of MGAT3



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Fig. 4 Expression of Recombinant MGAT3

A. Immunoblot with Anti-FLAG IgG

The Western blot shows protein expression levels across three lanes: Wild Type, MGAT3, and DGAT2. The y-axis is labeled 'kDa' and has two markers: 50 and 36. In the Wild Type lane, a prominent band is visible at approximately 50 kDa. In the MGAT3 lane, a band is visible at approximately 36 kDa. In the DGAT2 lane, a band is visible at approximately 50 kDa.

B. TLC MGAT Enzyme Assay

Exogenous Substrates	[¹⁴ C]Oleoyl-CoA		
	None	2-MAG	DAG

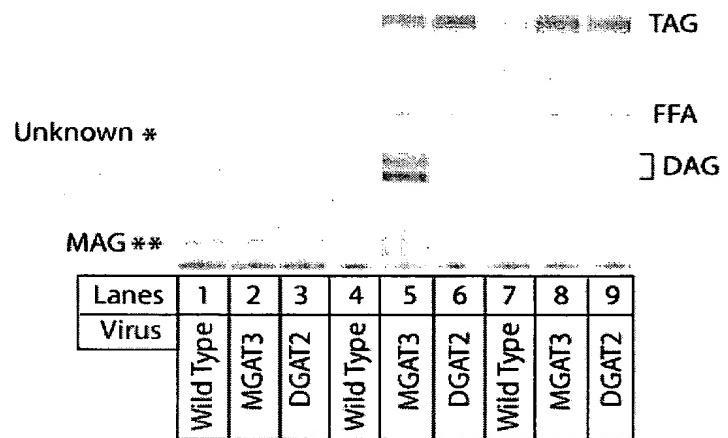


Fig.5 Time Course of Expression**A. Immunoblot with Anti-FLAG IgG**

Time (hr)	0	24	48	72
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MGAT3

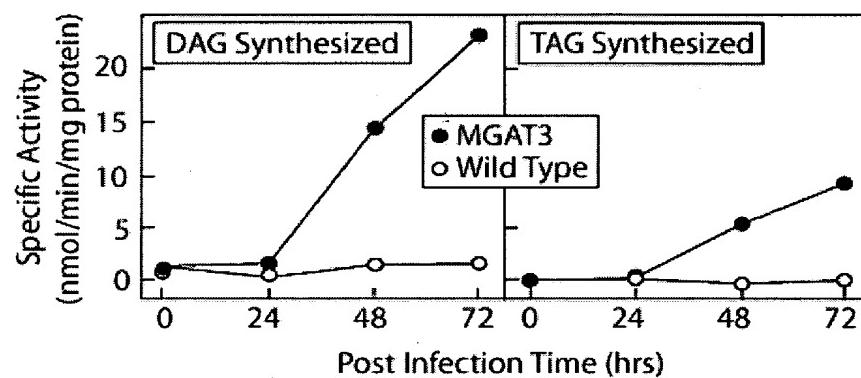
B. TLC Enzyme Assay

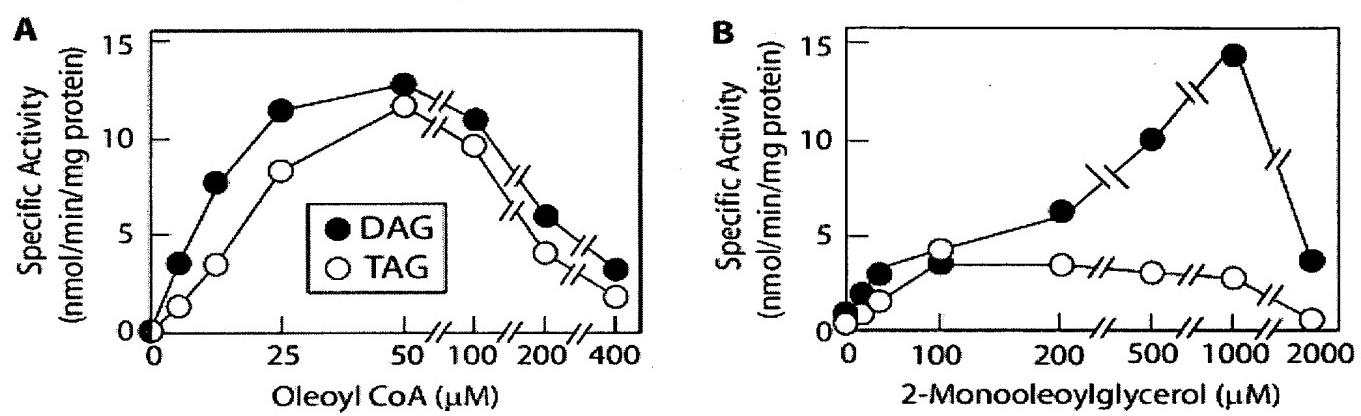
Fig. 6 Substrate Concentration Curve

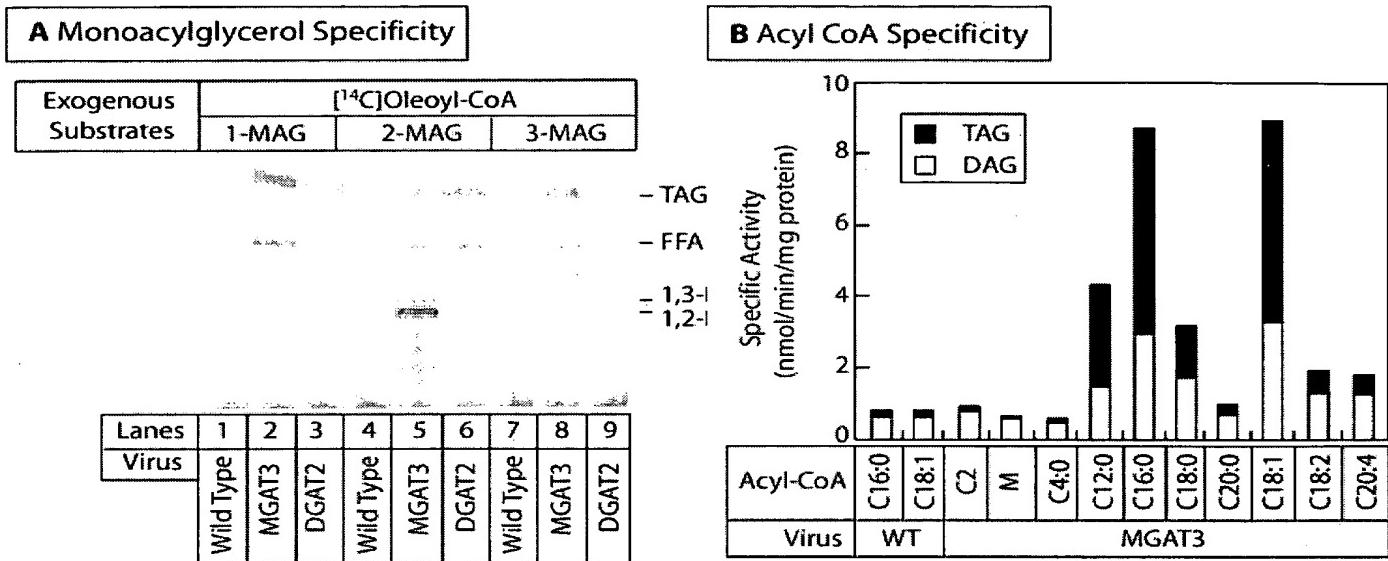
Fig. 7 Substrate Specificity

Fig.8 Relative Expression of MGAT3 in Normal Tissues

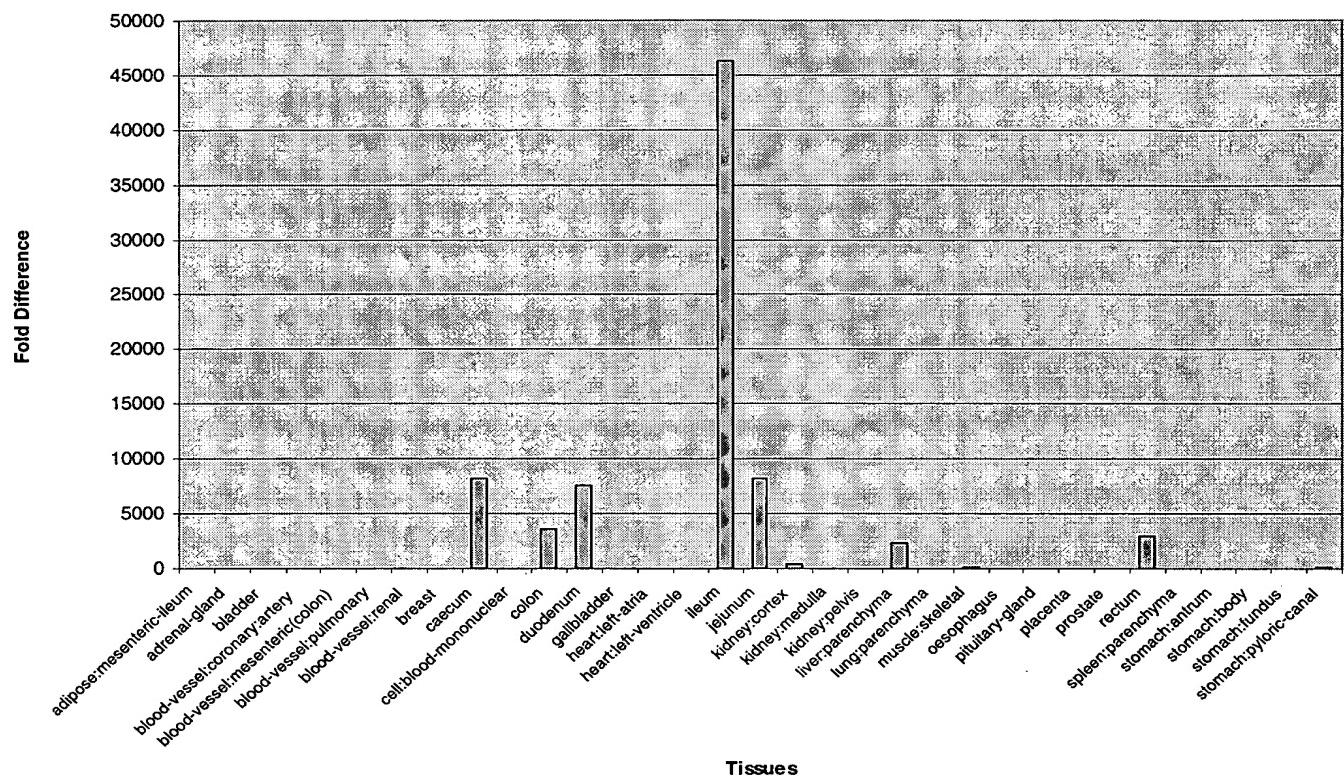


Fig.9 Relative expression of MGAT3 in Crohn's and control Ileum

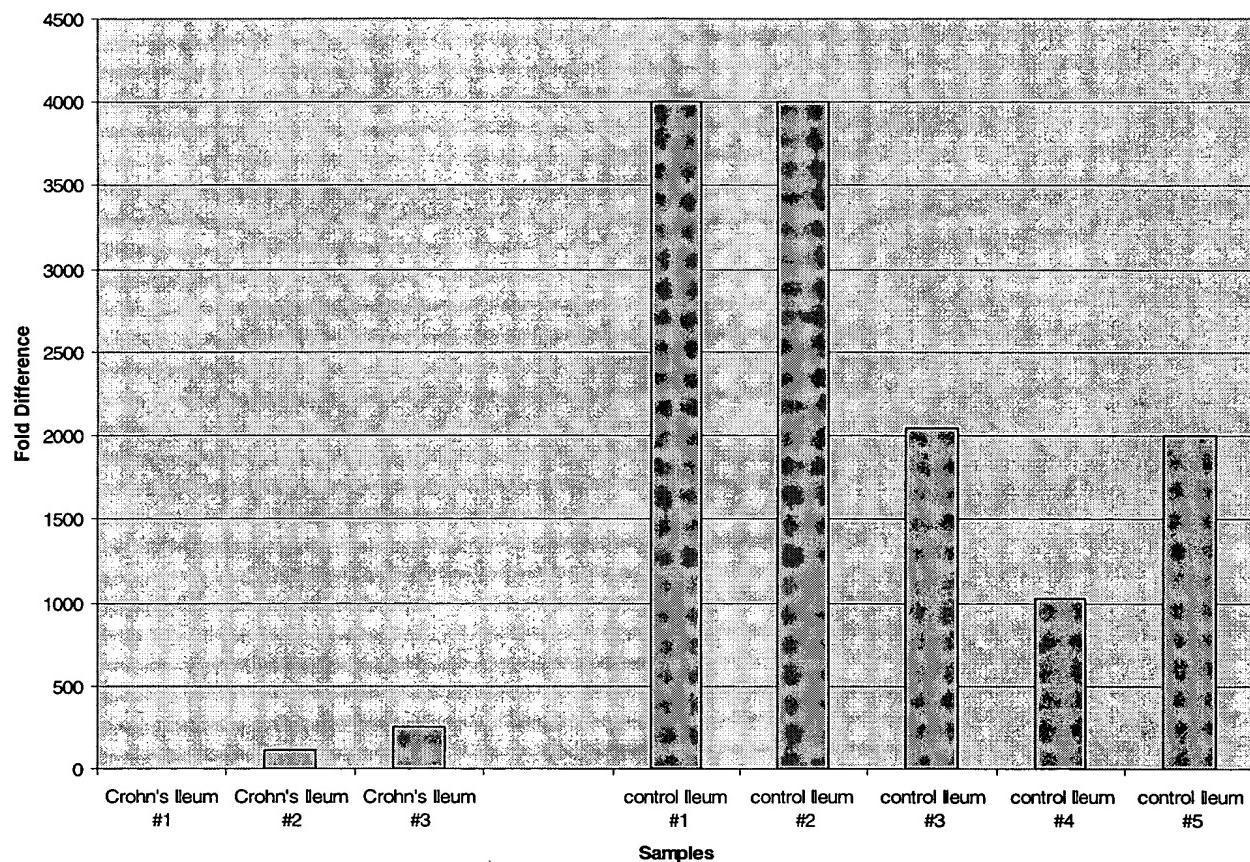


Fig.10 MGAT3 Gene is Located on Chromosome 7q22.1**□ Chromosome 7****□ Overview**